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Research Article

Cold as Ice: a novel eradication and control method for invasive Asian clam, *Corbicula fluminea*, using pelleted dry ice

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This study was contributed in relation to the 20th International Conference on Aquatic Invasive Species held in Fort Lauderdale, Florida, USA, October 22–26, 2017 (<http://www.icaais.org/html/previous20.html>). This conference has provided a venue for the exchange of information on various aspects of aquatic invasive species since its inception in 1990. The conference continues to provide an opportunity for dialog between academia, industry and environmental regulators.

Abstract

Eradication and control measures for invasive aquatic organisms require innovative methods that maximise efficacy whilst minimising environmental damage. Such methods should also ideally utilise readily available materials and have a relatively straightforward field application. The Asian clam, *Corbicula fluminea* (Müller, 1774) is a high impact freshwater invader that can dominate macroinvertebrate communities and physically alter benthic habitats. Moreover, *C. fluminea* has shown a high degree of physiological and ecological plasticity, and has displayed a remarkable capacity for human-mediated passive dispersal. Globally, despite repeated efforts to mitigate spread and implement substantial population control measures, *C. fluminea* continues to invade and spread. Accordingly, effective population eradication and control measures are urgently required. Here, we examine the efficacy of commercially available dry ice (DI) pellets (i.e. solid CO₂ pellets at –78 °C) to kill *C. fluminea*, when applied both directly (water absent) and indirectly (clams submerged). Experiment 1 revealed the ability of 9 mm DI pellets to induce substantial *C. fluminea* mortality, with a direct application of 300 g DI at 5 min exposure inducing 100% clam mortality. In experiments 2 and 3, DI pellets of 9 mm induced higher clam mortality than 3 mm pellets, DI slices and mixed DI pellet sizes (3 and 9 mm) at simulated clam densities of 1179 and 3930 individuals m⁻², especially when clams were submerged. Experiments 4 and 5 showed that DI application was highly effective even with clams that were covered in gravel or mud, due to the freezing of their surrounding substrate. Accordingly, these results demonstrate that DI can potentially be used for effective, rapid response control and eradication of *C. fluminea* populations. Whilst promising, our laboratory results require scaling up to field application and examination of the effects of water current, substrate, increased water depth, and greater *C. fluminea* population densities.

Key words: invasive alien species, biosecurity, dry ice, thermal shock, population control, eradication

Introduction

Aquatic invasive species (AIS) are major components of global change and exhibit a variety of negative

ecological, evolutionary, and economic impacts in freshwater ecosystems (Sala et al. 2000; Simberloff et al. 2013; Sousa et al. 2014). Management options for substantial eradication and control of established

AIS populations are often complex, resource-intensive and costly endeavours (Caffrey et al. 2011b; Wittmann et al. 2012a, b; Caffrey et al. 2014; Sousa et al. 2014; Piria et al. 2017). However, even if the challenges of implementation can be overcome, many traditional control and eradication methods are not always effective, and can often negatively affect non-target species (Caffrey et al. 2014; Sousa et al. 2014). Accordingly, new and innovative methodologies that maximise extermination efficacy towards target species, whilst minimising broad scale environmental damage, are urgently required. Such methods should also ideally utilise materials that are commercially available and be relatively straightforward to apply in the field. For example, Caffrey et al. (2010) achieved significant local eradication of the invasive aquatic macrophyte *Lagarosiphon major*, and subsequent restoration of native macrophyte communities, utilising biodegradable jute matting as a benthic barrier in a natural freshwater lake.

Since the mid-20th century, the invasive Asian clam, *Corbicula fluminea* (Müller, 1774) (Bivalvia, Cyrenidae; formerly Corbiculidae), has become widespread across all major river basins in Europe and the Americas (McMahon 1999; Karatayev et al. 2007; Lucy et al. 2012; Colwell et al. 2017; Gama et al. 2017). Native to south-east Asia, with endemic morphotypes occurring in Australia and Africa, *C. fluminea* is regarded as a high impact freshwater invader, which can dominate macroinvertebrate communities and physically alter benthic habitats (McMahon 1982; Karatayev et al. 2007; Sousa et al. 2008). In particular, *C. fluminea* can modify community and ecosystem dynamics, for example, through nutrient cycling and energy flow, phytoplankton depletion, competition for resources and substrate modification (Karatayev et al. 2007; Sousa et al. 2008; Sousa et al. 2014). In addition, macrofouling of agricultural, municipal and raw water extraction systems, increased sedimentation rates, and the disruption of ecosystem regulating services, can represent a substantial economic burden (McMahon 1999; Karatayev et al. 2007).

Corbicula fluminea has shown a high degree of physiological and ecological plasticity, and has displayed a remarkable capacity for human-mediated passive dispersal (McMahon 2002; Sousa et al. 2008; Belz et al. 2012; Lucy et al. 2012; Coughlan et al. 2017b). Accordingly, despite repeated management efforts to reduce the spread of this AIS, *C. fluminea* invasions continue to advance across hydrologically unconnected freshwater systems globally (e.g. Caffrey 2010; Barbour et al. 2013; Caffrey et al. 2016). Moreover, recent distribution models indicate that the current rate of climate change will increase

suitable habitat availability and favour the expansion of *C. fluminea* into new river basins, especially at higher latitudes (Gama et al. 2017). Therefore, while effective preventative biosecurity measures have been developed to mitigate against the spread of juvenile *C. fluminea* and other invaders, *via* cleaning, disinfection and drying of equipment and clothing (i.e. Check, Clean, Dry: Barbour et al. 2013; Coughlan et al. 2018; Cuthbert et al. 2018), there is an urgent need to expedite eradication and control of established clam populations, and to have available a suite of tried and tested methods that can be applied rapidly on detection of new outbreaks (Colwell et al. 2017).

Globally, although extensive eradication and control experiments have been conducted on *C. fluminea* (Wittmann et al. 2012a, b), none have been successful in providing substantial long-term management of *C. fluminea* populations. For example, mechanical dredging methods, and utilisation of benthic barriers (e.g. polyethylene and rubber), can achieve a short-term reduction of both *C. fluminea* biomass and density (Wittmann et al. 2012a, b; Sheehan et al. 2014). However, these strategies remain expensive, labour-intensive, and can have detrimental impacts on native species, without achieving complete eradication of the targeted *C. fluminea* populations. Accordingly, innovative techniques for the eradication and control of *C. fluminea* remain an urgent requirement.

Here, in the laboratory and in simulated clam patches, we examine the efficacy of commercially available dry ice (DI) pellets (i.e. solid CO₂ pellets at -78 °C) to kill *C. fluminea*. We hypothesize that the extreme cold produced will induce thermal shock, resulting in substantial clam mortality. We assess several key experimental factors: DI pellet type (size, mass, shape); clam size range; density of clams; exposure time; volume of water; direct or indirect DI application, and the presence of typical substrate layers (gravel and mud).

Methods

Specimen collection and maintenance

Specimens of *Corbicula fluminea* were obtained from the River Barrow in the Republic of Ireland (52°29'15.11"N; 6°55'42.20"W; Figure 1A). *Corbicula fluminea* was first detected on the island of Ireland at this tidal, freshwater section of the River Barrow in April 2010 (Sweeney 2009; Caffrey et al. 2011a), where the clams are often exposed at low tide. Maximum densities of *circa* 18,000 ind. m⁻² have been recorded at this site (Sheehan et al. 2014). Specimens were cleaned on site with the use of a 4 mm sieve and transported in source water to Queen's Marine



Figure 1. (A) Tidally exposed bed of *Corbicula fluminea* on the River Barrow, St. Mullins, Ireland. (B) View of *C. fluminea* specimens during a direct application of dry ice. (C) View of *C. fluminea* specimens during an indirect application (i.e. with water present) of dry ice to. (D) View of *C. fluminea* specimens immediately post exposure to dry ice. Photo credits: Stephen Potts and Daniel Walsh.

Laboratory (QML), Portaferry, Northern Ireland. In the laboratory, specimens were maintained in aerated aquaria using locally sourced lake water, with the clams displaying normal feeding behaviour and excellent survival (*circa* 95 %). Experiments were performed in a controlled temperature room (11–13 °C) on a 12:12 hour light:dark schedule. Specimens were allowed to acclimatise to the laboratory for at least one week prior to experimentation. Moreover, only living and feeding specimens were selected for experimental work, i.e. selected specimens were observed opening and/or extending their muscular foot.

Experiment 1: Dry ice as a mechanism for inducing Corbicula fluminea mortality

Corbicula fluminea specimens were exposed to four quantities (50, 100, 200, or 300 g) of 9 mm DI pellets as well as a control (no DI application). Three clam size ranges, small (8–14.9 mm), medium

(15–20.9 mm) and large (21–32 mm) specimens, were independently exposed to DI for durations of one or five minutes. Direct (no water present) and indirect (2.5 litres of water, at 100 mm depth) applications of DI were examined ($n = 3$ replicates). Groups consisting of ten specimens, of a single size range only, were used as an experimental unit. Moreover, to control for any impact of DI pellet mass on specimen mortality (as opposed to thermal shock), 300 g (maximum mass of DI utilised) of clean fine gravel (15 mm stone chips) was applied to all non DI replicates (control $n = 3$ replicates).

Experimental groups, within the desired size ranges above, were selected from the aquaria and placed within cylindrical plastic containers of 234 mm (height) × 180 mm (diameter). For groups selected for indirect DI application, 2.5 litres of clean, dechlorinated tap water was added to the container. The desired mass of the DI pellets was weighed and immediately added to the appropriate container. DI

pellets were distributed over the entire base area of the container as evenly as possible (Figure 1B), or across the surface area of the water column (Figure 1C). After the various exposure times, specimens were immediately removed from the experimental container (Figure 1D). Any specimens embedded within the DI were removed by hand, using a small metal ice-pick and cool dechlorinated tap water (*circa* 6 °C). All specimens were returned to 600 ml of dechlorinated bubbled water (11–13 °C) for a 24 hr recovery period, after which mortality was assessed. Specimens were considered dead if they were gaping, or if they offered no resistance to being teased apart with tweezers and did not reclose (see Matthews and McMahon 1999).

Experiment 2: The effectiveness of varied, commercially available dry ice formations

Adult specimens (15–26 mm) were exposed to 350 g of DI in the form of either 3 mm or 9 mm pellets, or as a slice. Whole dry ice slices (70 mm L × 45 mm W × 25 mm H; *circa* 1 kg) were cut to an arbitrary length with a mass of 350 g. Two experimental clam densities, consisting of both 30 clams (1179 ind. m⁻²) and 100 clams (3930 ind. m⁻²), were examined. Clams were exposed to DI for either 5, 15 or 30 minutes. Both direct (no water present) and indirect (2.5 litres of water, at a 100 mm depth) applications of DI were examined (*n* = 5 replicates). The required mass of DI pellets was weighed and immediately added to the appropriate container. As before, once the desired exposure time was obtained, both DI and specimens were immediately removed from experimental containers. All specimens were then left to recover for 24 hrs, after which mortality was assessed, as above.

Experiment 3: Combined application of selected commercially available dry ice pellets

Both 3 mm and 9 mm DI pellets induced high levels of *C. fluminea* mortality (see Results). Accordingly, a combined application of these DI pellet types was examined. Adult specimens (15–26 mm) were exposed to 400 g of DI composed of either 3 mm or 9 mm pellets alone, an even division (50:50; i.e. 200 g of each), or two parts one pellet type (267 g) to one part the remainder pellet type (133 g) (i.e. 2:1 and 1:2). In all pellet combinations, to increase the potential for 3 mm pellets to bind to clams, 3 mm pellets were always added to the experimental containers first, with heavier 9 mm added immediately afterwards. A clam density of 100 specimens (2930 ind. m⁻²) was examined. Clams were exposed to DI for 5, 15 or 30 minutes, for both direct and indirect (2.5 litres

of water) applications (*n* = 3 replicates). Living specimens were placed within the cylindrical experimental containers. Once the prescribed exposure time had elapsed, the specimens were immediately removed. Specimens were then left for a recovery period of 24 hrs, after which mortality was assessed as above.

Experiment 4: Dry ice application to Corbicula fluminea residing upon, within, and fully covered by fine gravel

Adult specimens (15–26 mm) were exposed to 400 or 600 g of 9 mm DI pellets. Specimens were all positioned upon a layer of gravel, were partially covered by a second layer of gravel, or were fully covered by the addition of a third layer of gravel. A clam density of 30 specimens (1179 ind. m⁻²) was examined. Clams were exposed to DI for either 15 or 30 minutes, for both direct and indirect (2.5 litres) applications (*n* = 3 replicates). A 350 g layer of clean fine gravel (15 mm stone chips) was evenly spread to cover the base of the experimental containers. Living specimens were placed directly on top of this gravel layer. A second layer of gravel (350 g) was then added to the required containers, and evenly spread to leave all specimens partially covered. A third layer of gravel (350 g) was added to the appropriate containers, and evenly spread to fully cover all specimens. After the prescribed DI exposure time, specimens were immediately removed, with mortality assessed after a 24 hr recovery period, as above.

Experiment 5: Dry ice application to Corbicula fluminea residing within mud layers

Adult specimens (15–26 mm) were exposed to 400 g of 9 mm DI pellets. Specimens were randomly mixed into a single (*circa* 800 g) or double (*circa* 1600 g) mud layers. A clam density of 30 specimens (1179 ind. m⁻²) was examined. Clams were exposed to a direct application of DI for the duration of 15 or 30 minutes (*n* = 3 replicates). Controls were established for the longer exposure time of 30 minutes only (control *n* = 3 replicates).

A mud layer was created by mixing 500 g earth, 200 g clean fine gravel (15 mm stone chips) and 100 ml of clean tap water. Living clam specimens were placed into this mud and randomly mixed through the substrate. A second mud layer was then added, if required, and all contents were thoroughly mixed. The mud and clam mixtures were then evenly spread on the base of the experimental containers, prior to the addition of DI pellets. After the prescribed DI exposure time, five litres of clean tap water (*circa* 6 °C)

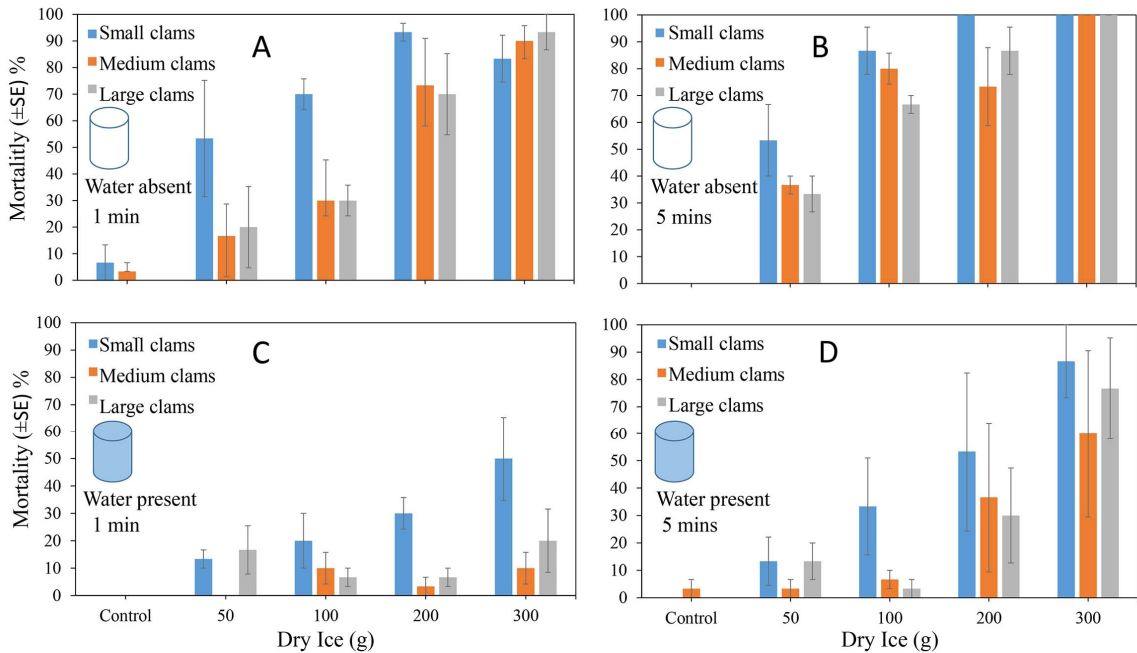


Figure 2. Mean mortality (± SE) of *Corbicula fluminea* specimens post exposure to various quantities of 9 mm dry ice pellets ($n = 3$). Size ranges: small (8–14.9 mm); medium (15–20.9 mm); and large (21–32 mm), were utilised. All size ranges were exposed to dry ice for either a one (A and C) or five (B and D) minute time period. A and B = 0 litres, 0 mm (h) (i.e. direct application), C and D = 2.55 litres, 100 mm (h) (i.e. indirect application) of water.

was added to each container and allowed to stand for a 20 minute period. Specimens were then immediately removed, with mortality assessed as above following a 24 hr recovery period.

Statistical Analysis

All data were analysed in R version 3.3.3 (R Core Team 2017) with Generalised Linear Models (GLMs). As residual deviance was greater than the degrees of freedom, quasi-Poisson error distributions were used to account for over-dispersion of residuals and to analyse the numbers of dead *C. fluminea* in each experiment with respect to each treatment term and associated interactions.

Results

Experiment 1: Dry ice as a mechanism for inducing *Corbicula fluminea* mortality

There was 87–100% survival in control clams groups, with between 0–100 % mortality among DI-exposed clams (Figure 2). There was significantly higher clam mortality with greater quantities of DI, for smaller clams, at the longer exposure time, and with direct (no

water) application (all $P < 0.001$; Figure 2, Table 1). The significant interaction terms (Table 1) reflect the greater increases in clam mortality at higher DI mass, for smaller clams, at the longer exposure time, and when water was absent (see Figure 2).

Experiment 2: The effectiveness of varied, commercially available dry ice formations

There was 88–99 % survival in control clam groups, with between 1–100 % mortality of DI exposed clams recorded from this experiment (Figure 3). There was significantly different clam mortality with the varied DI formations, and greater mortality rates at the lower clam density, and with direct application (all $P < 0.001$; Figure 3, Table 2). In general, longer exposure times also resulted in increased mortality ($P < 0.001$; Figure 3, Table 2). The significant interaction terms (Table 2) reflect increases in clam mortality between varied DI formations, for the lower density, for the longer exposure times, and with direct application (see Figure 3). In particular, applications of DI pellets (3 mm and 9 mm) resulted in greater *C. fluminea* mortality than the addition of a DI slice, and 9 mm pellets were clearly the most effective at killing clams (Figure 3).

Table 1. Quasi-Poisson Generalised Linear Model (GLM) performed on the number of dead *Corbicula fluminea* specimens for Experiment 1: Dry ice as a mechanism for inducing *Corbicula fluminea* mortality. DI = Control, 50, 100, 200, or 300 g of 9 mm DI pellets. Exposure time = 1 or 5 min. Size ranges = small (8–14.9 mm), medium (15–20.9 mm), or large (21–32 mm) specimens. Water = (1) water volume (0 or 2.55 litres), (2) water depth (0 or 100 mm), and (3) direct or indirect DI application. NS = non-significant; $\alpha = 0.05$.

Source of variation	<i>F</i>	<i>P</i>
Dry Ice (DI)	$F_{4, 175} = 92.3813$	$P < 0.001$
Size Range (Size)	$F_{2, 171} = 10.8945$	$P < 0.001$
Exposure Time (Time)	$F_{1, 174} = 22.8653$	$P < 0.001$
Water (i.e. volume, depth, and DI application)	$F_{1, 173} = 148.1147$	$P < 0.001$
DI \times Size	$F_{8, 154} = 1.3777$	NS
DI \times Time	$F_{4, 167} = 0.7552$	NS
DI \times Water	$F_{4, 163} = 3.8098$	$P < 0.01$
Size \times Time	$F_{2, 152} = 1.3502$	NS
Size \times Water	$F_{2, 150} = 5.1157$	$P < 0.01$
Time \times Water	$F_{1, 162} = 10.6196$	$P < 0.01$
DI \times Size \times Time	$F_{8, 138} = 0.3786$	NS
DI \times Size \times Water	$F_{8, 130} = 1.0582$	NS
DI \times Time \times Water	$F_{4, 146} = 3.4009$	$P < 0.05$
Size \times Time \times Water	$F_{2, 128} = 1.1009$	NS
DI \times Size \times Time \times Water	$F_{8, 120} = 1.1299$	NS

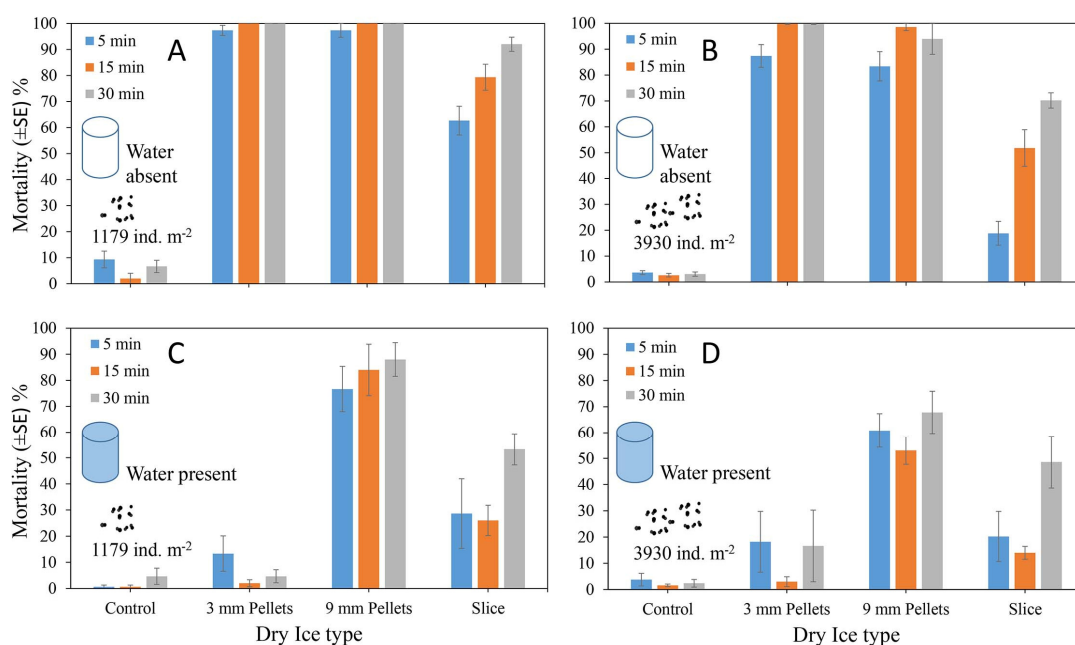


Figure 3. Mean mortality (\pm SE) of 30 adult *Corbicula fluminea* specimens (1179 ind. m⁻²) (A and C) and 100 adult specimens (3930 ind. m⁻²) (B and D), 24 hrs post varied exposure times to 350 g of different dry ice formations ($n = 5$). A and B = 0 litres, 0 mm (h) (i.e. direct application), C and D = 2.55 litres, 100 mm (h) (i.e. indirect application) of water. Black dots highlight the two specimen densities.

Experiment 3: Combined application of selected commercially available dry ice pellets

There was 79–98% survival in control clam groups, with between 8.5–100% mortality of DI exposed clams in this experiment (Figure 4). There was significantly different clam mortality with the various DI

combinations ($P < 0.001$), and greater mortality at longer exposure times ($P < 0.01$), and with direct application ($P < 0.001$; Figure 4A, Table 3). The significant interaction terms (Table 3) reflect the enhanced clam mortality between the varied DI combinations, for the longer exposure times, and with direct application (see Figure 4). In particular,

Table 2. Quasi-Poisson Generalised Linear Model (GLM) performed on the number of dead *Corbicula fluminea* specimens for Experiment 2: The effectiveness of varied, commercially available dry ice formations. DI = Control, 3 or 9 mm pellets, or as a DI slice (see methods section). Exposure Time = 5, 15 or 30 min. Clam Density = groups of 30 (i.e., 1178.9 ind. m⁻²) or 100 (3929.7 ind. m⁻²) adult specimens. Water = (1) water volume (0 or 2.55 litres), (2) water depth (0 or 100 mm), and (3) direct or indirect DI application. NS = non-significant; α = 0.05.

Source of variation	<i>F</i>	<i>P</i>
Dry Ice (DI)	$F_{3, 236} = 265.2584$	$P < 0.001$
Clam density (Density)	$F_{1, 233} = 359.7943$	$P < 0.001$
Exposure Time (Time)	$F_{2, 234} = 10.1459$	$P < 0.001$
Water (i.e. volume, depth, and DI application)	$F_{1, 232} = 243.9251$	$P < 0.001$
DI \times Density	$F_{3, 223} = 2.7632$	$P < 0.05$
DI \times Time	$F_{6, 226} = 5.4814$	$P < 0.001$
DI \times Water	$F_{3, 218} = 49.9436$	$P < 0.001$
Density \times Time	$F_{2, 221} = 1.0855$	NS
Density \times Water	$F_{1, 215} = 0.0635$	NS
Time \times Water	$F_{2, 216} = 9.0548$	$P < 0.001$
DI \times Density \times Time	$F_{6, 209} = 0.7059$	NS
DI \times Density \times Water	$F_{3, 200} = 2.3822$	NS
DI \times Time \times Water	$F_{6, 203} = 2.3693$	$P < 0.05$
Density \times Time \times Water	$F_{2, 198} = 1.0693$	NS
DI \times Density \times Time \times Water	$F_{6, 192} = 0.5139$	NS

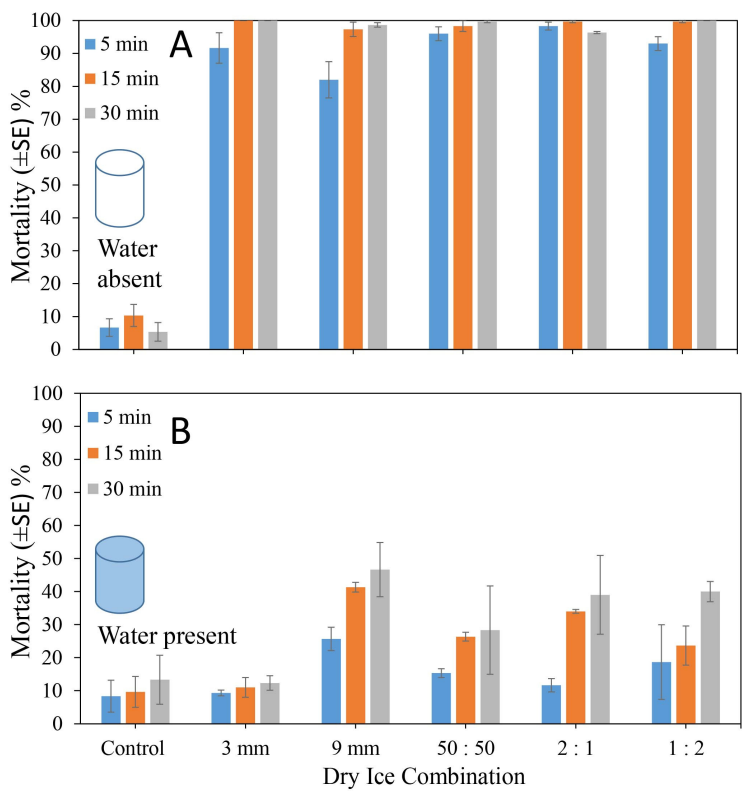


Figure 4. Mean mortality (\pm SE) of 100 adult *Corbicula fluminea* specimens (3930 ind. m⁻²) 24 hrs post varied exposure times to 400 g of different dry ice pellet combinations (n = 3). Dry ice applications were composed of either 3 mm or 9 mm pellets alone, an even division (50:50; i.e. 200 g of each), or two parts one pellet type (267 g) to one part the remainder pellet type (133 g) (i.e. 2:1 and 1:2); The 3 mm pellets were always applied first, and immediately followed by 9 mm pellets. A = 0 litres, 0 mm (h) (i.e. direct application), B = 2.55 litres, 100 mm (h) (i.e. indirect application) of water.

unmixed 9 mm pellets resulted in the highest clam mortality during indirect applications, whilst the application of 3 mm pellets, mixed or unmixed, enhanced efficacies during direct applications under shorter exposure times (Figure 4).

Experiment 4: Dry ice application to C. fluminea residing upon, within, and fully covered by fine gravel

There was 83–94 % survival in control clam groups, with between 31–100 % mortality of DI exposed clams

Table 3. Quasi-Poisson Generalised Linear Model (GLM) performed on the number of dead *Corbicula fluminea* specimens for Experiment 3: Combined application of selected commercially available dry ice pellets. DI = Control, 3 or 9 mm pellets alone, an even division (50:50), or two parts one pellet type to one part the remainder pellet type (2:1 or 1:2). Exposure Time = 5, 15 or 30 min. Water = (1) water volume (0 or 2.55 litres), (2) water depth (0 or 100 mm), and (3) direct or indirect DI application. NS = non-significant; $\alpha = 0.05$.

Source of variation	<i>F</i>	<i>P</i>
Dry Ice (DI)	$F_{5, 102} = 76.1479$	$P < 0.001$
Exposure Time (Time)	$F_{2, 100} = 6.7121$	$P < 0.01$
Water (i.e. volume, depth, and DI application)	$F_{1, 99} = 629.5575$	$P < 0.001$
DI \times Time	$F_{10, 89} = 0.2229$	NS
DI \times Water	$F_{5, 84} = 15.4678$	$P < 0.001$
Time \times Water	$F_{2, 82} = 9.7469$	$P < 0.001$
DI \times Time \times Water	$F_{10, 72} = 0.8285$	NS

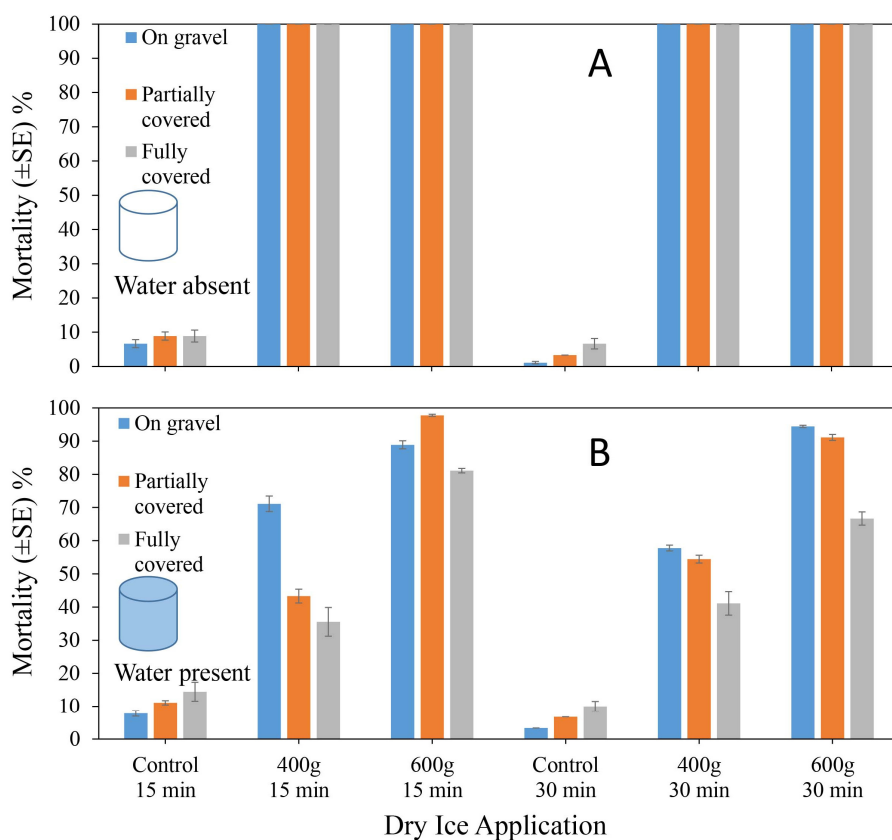


Figure 5. Mean mortality (\pm SE) of 30 adult *Corbicula fluminea* specimens (1179 ind. m⁻²) 24 hrs post varied exposure times to either 400 or 600 g of 9 mm dry ice pellets ($n = 3$). Specimens were either placed directly (1) upon a gravel layer; (2) were partially covered; or (3), were fully covered by additional gravel layers prior to dry ice application. A = 0 litres, 0 mm (h) (i.e. direct application), B = 2.55 litres, 100 mm (h) (i.e. indirect application) of water.

in this experiment (Figure 5). There was no overall significant difference in clam mortality with respect to gravel coverage, although interaction terms suggest some reduction of efficacy of DI due to gravel and higher efficacy with the larger mass of DI when the clams were covered by water (Figure 5B; Table 4). There was significantly higher clam mortality with the greater quantity of DI, and with direct application (both $P < 0.001$; Figure 5, Table 4). The significant interaction terms (Table 4) reflect the more

dramatic increases in clam mortality with greater quantity of DI, and with direct application (see Figure 5).

Experiment 5: Dry ice application to *Corbicula fluminea* residing within mud layers

There was 95–100% survival in control clam groups, with between 5.9–100 % mortality of DI exposed clams in this experiment (Figure 6). The significant

Table 4. Quasi-Poisson Generalised Linear Model (GLM) performed on the number of dead *Corbicula fluminea* specimens for Experiment 4: Dry ice application to *C. fluminea* residing upon, within, and fully covered by fine gravel. DI = Control, 400 or 600 g of 9 mm DI pellets. Exposure Time = 15 or 30 min. Gravel = specimens sitting upon a gravel layer, or partially, or fully covered by additional gravel layers. Water = (1) water volume (0 or 2.55 litres), (2) water depth (0 or 100 mm), and (3) direct or indirect DI application. NS = non-significant; $\alpha = 0.05$.

Source of variation	<i>F</i>	<i>P</i>
Dry Ice (DI)	$F_{2,105} = 561.5255$	$P < 0.001$
Gravel (upon a gravel layer, partially or fully covered)	$F_{2,101} = 1.7628$	NS
Exposure Time (Time)	$F_{1,104} = 0.7166$	NS
Water (i.e. volume, depth, and DI application)	$F_{1,103} = 61.3259$	$P < 0.001$
DI \times Gravel	$F_{4,92} = 2.8732$	$P < 0.05$
DI \times Time	$F_{2,99} = 3.8215$	$P < 0.05$
DI \times Water	$F_{2,97} = 23.6612$	$P < 0.001$
Gravel \times Time	$F_{2,90} = 0.0862$	NS
Gravel \times Water	$F_{2,88} = 4.5098$	$P < 0.05$
Time \times Water	$F_{1,96} = 0.0239$	NS
DI \times Gravel \times Time	$F_{4,82} = 0.9043$	NS
DI \times Gravel \times Water	$F_{4,78} = 0.7727$	NS
DI \times Time \times Water	$F_{2,86} = 0.3090$	NS
Gravel \times Time \times Water	$F_{2,76} = 0.2056$	NS
DI \times Gravel \times Time \times Water	$F_{4,72} = 0.7330$	NS

Figure 6. Mean mortality (\pm SE) of 30 adult *Corbicula fluminea* specimens (1179 ind. m⁻²) 24 hrs post varied exposure times to a direct application of 400 g of 9 mm dry ice pellets ($n = 3$). Specimens were all randomly mixed into a single (circa 800 g) or double (circa 1600 g) mud layers, prior to dry ice application.

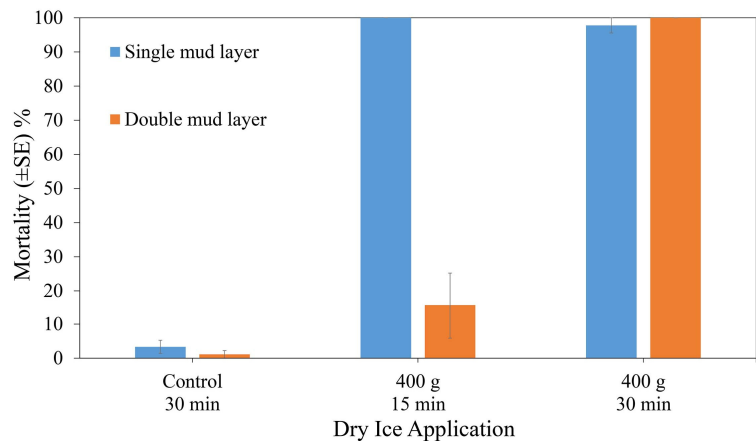


Table 5. Quasi-Poisson Generalised Linear Model (GLM) performed on the number of dead *Corbicula fluminea* specimens for Experiment 5: Dry ice application to *C. fluminea* residing within mud layers. DI = Control or 400 g of 9 mm pellets. Exposure Time = 15 or 30 min. Mud Layers = single (circa 800 g) or double (circa 1600 g). NS = non-significant; $\alpha = 0.05$. n/a = non-calculable statistic.

Source of variation	<i>F</i>	<i>P</i>
Dry Ice (DI)	$F_{1,16} = 157.837$	$P < 0.001$
Exposure Time (Time)	$F_{1,15} = 15.7726$	$P < 0.01$
Mud Layers (Mud)	$F_{1,14} = 16.4090$	$P < 0.01$
DI \times Time	n/a	n/a
DI \times Mud	$F_{1,13} = 0.2037$	NS
Time \times Mud	$F_{1,12} = 34.0177$	$P < 0.001$
DI \times Time \times Mud	n/a	n/a

main and interaction terms show that there was very high efficacy of DI application even when clams were encased in mud, especially at the longer exposure time (Figure 6; Table 5).

Discussion

Experiment 1 revealed that application of dry ice (DI), as solid CO₂ pellets at -78°C , is an effective method for inducing *C. fluminea* mortality. Greater

DI quantities and direct (no water present) applications resulted in higher mortality, with smaller clams being more susceptible to thermal shock than larger specimens (see also Werner and Rothhaupt 2008). In particular, direct application of 300 g DI at 5 min exposure induced 100% mortality across all size ranges. Further, Experiment 2 demonstrates that 9 mm pellets induced higher clam mortality than either 3 mm pellets and DI slices, at simulated clam densities of 1179 and 3930 ind. m⁻², especially for indirect (clams submerged in water) applications. During indirect applications, while 3 mm DI pellets initially sank to the bottom of the water column, a large proportion of these pellets often rapidly consolidated into a single ice mass and floated to the top of the water column. However, 9 mm DI pellets mostly remained at the benthic level of the water column, partially encapsulating *C. fluminea* specimens. The encapsulation of *C. fluminea* by 9 mm pellets is likely have increased the effectiveness of DI as a mechanism to induce mortality since a greater surface area of the DI was in contact with the shell of the clams. Strategies to ensure that the DI pellets remain in contact with the river bed and clams, thereby increasing this surface area effect, might further increase the efficacy of DI in killing clams. Consideration to applying jute matting (see Caffrey et al. 2010) immediately following DI pellet application will be given during further studies.

Experiment 3 has further shown that 9 mm DI pellets can induce higher clam mortality than mixed DI pellet sizes. Once again, during indirect DI applications, 3 mm pellets were observed to float to the surface of the water column. However, this loss of 3 mm pellets from the benthic level appeared to be reduced by the addition of 9 mm pellets in combined DI pellet applications. While 9 mm pellets were more effective, combined applications of 3 and 9 mm pellets merit further investigation, especially with the use of jute matting and other strategies to increase the surface area effects, as discussed above. In addition, direct application of both 3 and 9 mm pellets to the underwater benthic layer, particularly in realistic field scenarios with the presence of substrate, need to be examined.

Experiments 4 and 5 show that 9 mm DI pellet application was highly effective in inducing clam mortality, even those that were buried in gravel or mud. While *C. fluminea* generally prefers sandier sediments mixed with silt and clay, this species can be found in all types of sediments (Sousa et al. 2008). For example, in the River Barrow, *C. fluminea* resides in sediment dominated by sands and gravel (Sheehan et al. 2014). Crucially, DI tended to bind *C. fluminea* to both the gravel and mud, creating a layer of frozen

substrate around the specimens, particularly during indirect applications. This enhanced encapsulation of *C. fluminea* appears to have increased the efficacy of DI, rather than provide an insulative effect against thermal shock.

Overall, when taken together, our five experiments demonstrate that DI applications can induce substantial *C. fluminea* mortality. In general, greater quantities of DI applied as direct applications, with longer exposure times, often resulted in complete mortality of targeted adult clam specimens. While the indirect application of DI through a water column significantly reduced mortality rates across all five experiments, utilisation of greater quantities of DI will likely increase this mortality rate.

The ability of DI-induced thermal shock to eradicate or control other sedentary (e.g. zebra mussels *Dreissena polymorpha* Pallas, 1771; quagga mussels *Dreissena rostriformis bugensis* Andrusov, 1897; golden mussel *Limnoperna fortunei* Dunker, 1857) or low mobility AIS, such as gastropoda, also requires exploration. In particular, early application of DI to New Zealand mud snail (*Potamopyrgus antipodarum* Gray, 1843) infestations, an emerging AIS across North America that can attain densities of up to 500,000 ind. m⁻² (Hall et al. 2003), may reduce invader spread and impact. Equally, the potential for DI to mitigate against aquatic macrophytes (e.g. Nuttall's waterweed *Elodea nuttallii* [Planch.] H. St. John, 1920; Curly waterweed *Lagarosiphon major* [Ridley] Moss, 1928) should be considered.

While many mobile non-target species, such as fish, will likely rapidly flee a DI treatment area and the effects of thermal shock, DI application will presumably induce mortality among less mobile species. Moreover, DI-induced thermal shock and associated lowering of water pH (i.e. acidification) might result in a degree of invertebrate drift and mortality (Eriksen et al. 2009; Kjærstad and Arnekleiv 2011). However, given the biological connectivity and rapid species recolonization times associated with lotic systems (Yount and Niemi 1990; Caffrey et al. 2010; Wittmann et al. 2012a,b; Coughlan et al. 2017a), DI application may be a suitable management tool that will be followed by rapid recovery. In particular, invertebrate assemblages essential for supporting higher trophic levels within localised habitats have previously been observed to be resilient to the conservative deployment of pesticides (e.g. rotenone) for AIS control, when upstream sources of recruitment exist (Bellingan et al. 2015). For example, a variety of Amphipoda, Chironomidae, Oligochaeta, Gastropoda (Planorbidae and Physidae), Ceratopogonidae and Hydracarinae species have been observed to rapidly recolonise (3–12 months) post cessation of *in*

situ experimental anoxia treatments (Wittmann et al. 2012b). Furthermore, short-term negative impacts associated with invader eradication will likely be heavily outweighed by the long-term positive conservation benefits gained by removing damaging AIS (Woodford et al. 2013). However, although the sedentary nature of clams and more mobile capacity of most other organisms will mitigate non-target effects, the impact of DI on non-target organisms and aquatic habitats will need to be explored.

Finally, DI will rapidly dissipate into gaseous carbon dioxide once exposed to ambient air and water temperatures. Therefore, DI applications may be preferable to mechanical control methods (e.g. harvesting by suction or other dredging methods, and benthic barriers), which can result in habitat alteration, or to chemical treatments, with the potential for detrimental impact and lingering effect on non-target taxa (Sousa et al. 2014). However, despite apparent invader control or eradication success, prediction of a long-term population response to any management strategy can be challenging. In particular, a single self-fertilising *C. fluminea* specimen can, over-time, result in a substantial population (McMahon 2002). Therefore, only long-term monitoring will truly reveal the impacts of any control methodology (Wittmann et al. 2012b).

Overall, the results presented here demonstrate that DI can potentially be used for effective, rapid response control and eradication of *C. fluminea* populations, both on tidally exposed river beds and on clams residing underwater. However, additional research is required to assess the effect of *in situ* factors such as water current, substrate, increased water depth, and greater *C. fluminea* population densities. While we have examined DI application as an innovative eradication and control method for *C. fluminea*, only mortality rates 24 hrs post-exposure have been considered in the present study. Future research should examine potential sub-lethal effects upon *C. fluminea*, other invaders and non-target organisms (e.g. reduced growth or reproductive output, acute or chronic morbidity), which may possibly be induced by DI application alone, or synergistically with other control and eradication actions. Furthermore, examination of synergistic mechanisms to increase clam encapsulation with DI should include benthic barriers to retain DI against the benthic layer (e.g. jute matting), and mechanisms for direct delivery of DI to the underwater benthic layer (e.g. pump applications). Moreover, the effect of substrate to enhance DI treatment should be further explored. Equally, the effect of other sediment types (e.g. sand and gravel mixtures), *C. fluminea* burrowing behaviour, and the

impact of greater clam population densities, will all need to be thoroughly examined in the context of DI applications. In addition, the effect of raking, dredging or furrowing upon clam beds, to disrupt the integrity of the benthic layer prior to DI application, merits investigation. Finally, to be truly effective, multiple DI applications may be required to deliver eradication or substantial population control. Given the current paucity of effective, environmentally friendly, rapid response invader eradication and control protocols, the apparent excellent potential of DI applications must be further explored.

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